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PRINCIPAL INVESTIGATOR: V. Reggie Edgerton, Ph.D.

CONTRACTING ORGANIZATION: University of California, Los Angeles
Los Angeles, CA 90095

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14. ABSTRACT
We have designed and fabricated flexible electrode array system and demonstrated that the epidural stimulating arrays work better and can facilitate locomotion significantly earlier after spinal cord injury than conventional wire epidural stimulating electrodes. We have been able to maintain a functional, implanted array in a complete spinal rat for 6 weeks. We found stable parameters of the electrode array (impedance of electrodes and thresholds of evoked responses) for this period and observed stable rhythmic hindlimb activity facilitated with stimulation from selected electrode combinations. We also found that stimulation with the epidural electrode array could selectively activate spinal pathways responsible for monosynaptic and polysynaptic responses that may provide new perspectives in the assessment of the spinal cord after injury.

15. SUBJECT TERMS
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Table of Contents

INTRODUCTION:

The main objective of this project is the development of new techniques to provide high quality behavioral outcome and electrophysiological analyses that will provide quantitative assessments of recovery of the spinal control of posture and locomotion after a spinal cord injury. The main hypotheses of this project were:

1. The Electrode Array (EA) System that we develop can be implanted over the lumbosacral spinal cord and be used in complete spinal rats for a) evoking monosynaptic and polysynaptic potentials in multiple muscles simultaneously, and b) facilitating bilateral weight-bearing stepping and further to gain an even higher level of performance in stepping when the rats are trained daily to step.
2. The EA System that we develop can be used to modulate and assess the level of efficacy of activation of flexors and extensors during stepping over the course of recovery after a complete SCI.
3. Stimulation with the developed EA can be used to more successfully generate weight-bearing stepping in complete spinal rats when combined with training than can be achieved with training alone or stimulation alone.

BODY:

Within one year we expected to have developed a first generation of an EA System and determined its effectiveness in enhancing the potential of ES as a means of improving the functionality of the spinal circuitry to facilitate stepping in SCI rats. We also expected to have determined the sensitivity of our stimulation procedures with respect to the density of the EA and to the spatial profile of the electrodes that are stimulated and demonstrate that the EA System is safe and durable for chronic implantation. In this process we expected to develop a 32-channel computer controlled stimulation device for maximizing the potential of our 32-electrode design. Finally we expected to have determined whether the anticipated improvement in stepping as a result of training can be best achieved by combining daily periods of ES in combination with step training.

We can state that all our objectives were reached, except the last one. We now have all required technologies and tools to complete the last goal and determine whether stepping can be best achieved by combining daily periods of ES with electrode array in combination with step training.

Aim 1: Develop a chronically implantable system for high density ES of the lumbosacral spinal cord.

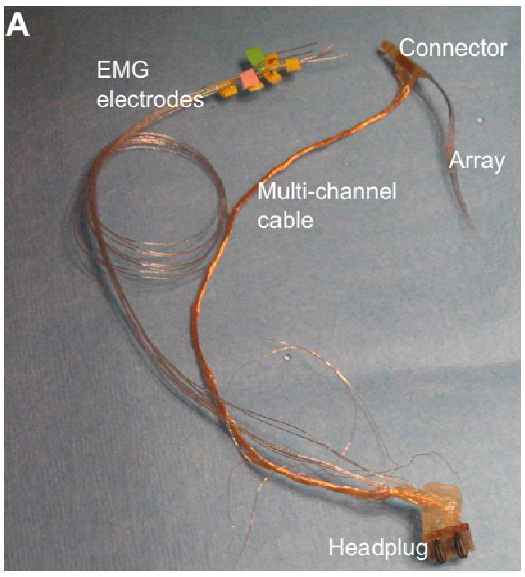
Aim 1.1: Design and fabricate a flexible, high-density EA System that can be implanted chronically over the lumbosacral spinal cord of the rat. These micro-fabricated EA's will consist of a flexible biocompatible Parylene-substrate embedded with flexible metal electrode pads (in a 9x3 pattern) and flexible metal connecting wires.

1.1.1 We have designed and fabricated the next generation 3x9 flexible ES array system. The arrays are based on our existing flexible electrode technology with a parylene-platinum-parylene thin film multilayer microfabrication process. A single metal layer was found to be sufficient for meeting the requirements of the electrode array and the routing of traces, simplifying the fabrication. Photolithography is used to define the liftoff process for the metal traces and also for the mask during the oxygen plasma etching of parylene that defines the shape of the device and its array of electrodes. After separating the final multilayer devices from the support wafer, they are annealed in a vacuum oven to prevent delamination.

1.1.2. The arrays' mechanical and electrical properties were bench-top tested. In the first iteration of the array, the device was made long enough to span the distance between the headplug and spinal cord. Impedance was measured and found to be well below the limits necessary for stimulation and recording. However, it was found that the electromechanical properties of the device did not allow the platinum traces to maintain their electrical continuity when subjected to the vigorous motions of a moving animal for several weeks. In particular, the relative motion between the headplug and the entry point into the spinal column, and the inability to control the motion of soft tissues surrounding the device, caused excessive folding and creasing of the device and broke the metal traces. Bench-top testing showed that silicone protection of the array was not quite sufficient to protect it from experiencing critical levels of strain under similar conditions. In contrast, the portion of the array inside the spinal column and on top of the dura was subjected to much more limited motion, and no damage of the array was observed. For this reason, a shorter flexible array limited to the vicinity of the spinal cord of most interest was designed and used in the implantation scheme described in the following section.

Aim 1.2: Design and fabricate a flexible, high-density EA System that can be implanted chronically over the lumbosacral spinal cord of the rat. These micro-fabricated EA's will consist of a flexible biocompatible Parylene-substrate embedded with flexible metal electrode pads (in a 9x3 pattern) and flexible metal connecting wires.

1.2.1. A flexible ES array was successfully interfaced with multichannel connectors, an extension cable, and a multichannel headplug developed for the rat model. The implant uses a custom printed circuit board as a base-plate (this is fixed to the spine during surgery), and the required electrical connections to the short flexible array are made with silver epoxy. A bundle of Teflon-coated 75-micron diameter gold wire is connected between the spinal base-plate and the headplug, and the entire base-plate along with all connections are insulated with biocompatible epoxy. It is this bundle of wires, additionally coated with silicone, which handles the motion of the rat that damaged the earlier long flexible array. The headplug uses fine-pitch surface-mount connectors to provide 72 connections in a compact footprint, which provides enough connections for both the electrode array and EMG wires with room to spare. Additional connection capacity can be achieved with minimal changes in this design. Since the animal can occasionally move violently during testing, the headplug also was redesigned to include the ability to automatically disconnect from the external measurement cable to avoid damage. Using this system, it was found that most of the connections from the array to the external measurement and stimulation electronics – including the base-plate, bundle of wires, headplug, EMG wires, and measurement cable – were reliable for the duration of the experiments (Figure 1).



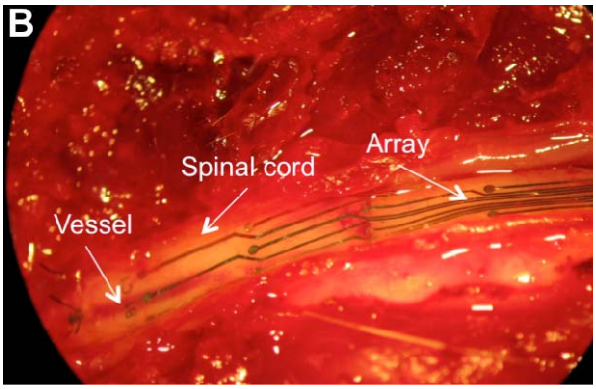


Figure 1: A. Multiple component electrode array system with parylene-based array, EMG electrodes, multi-channel cable, connector, and headplug. B. Parylene-based array after implantation on the spinal cord.

Assembly design failures and improvements.

Our current design that summarizes all of the improvements we have made based on our previous failures is shown in **Figure 2**. The design involves a micro-fabricated electrode array and hand-assembled components to address the various technical issues of a chronic implant in a rat. The electrode array is fabricated with a sandwich structure of parylene-metal-parylene. Parylene is a USP class VI biocompatible material, and its mechanical properties allow the necessary flexibility to make good epidural contact with the spinal cord. The metal layer is PVD-deposited platinum with a titanium adhesion layer, and 27 electrodes are arranged in 9 rows that are 3.3 mm apart with 3 electrodes in each row placed 1.0 mm apart. Other components in the assembly include a spinal baseplate to interface with the array, a custom head connector with high-density connectors, and a gold wire bundle to connect the head connector and baseplate. Medical grade epoxy and silicone are used to seal and strengthen the implant.

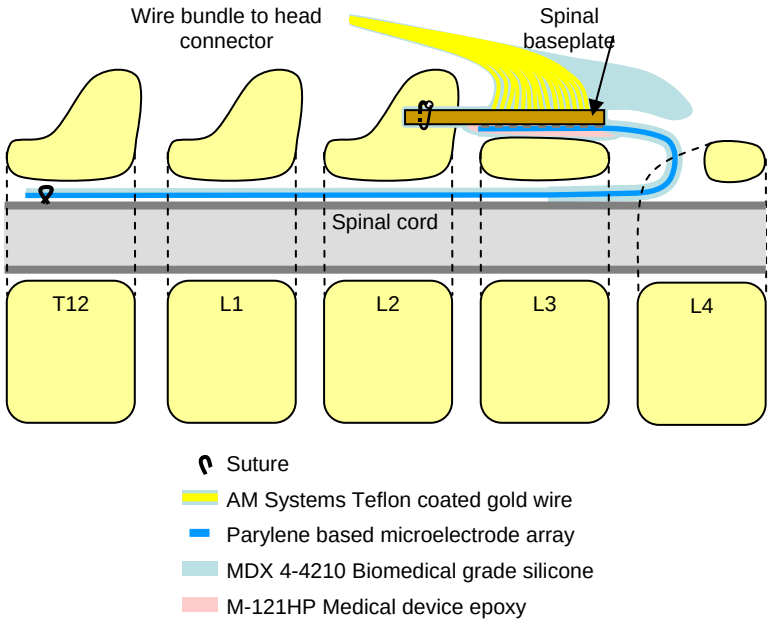


Figure 2. Current design of the chronic array implant.

In the first design of the spinal cord implant we attempted to use one thin film micro-fabricated device to cover the entire distance between the head connector and the spinal cord. In chronic experiments, however, the movement of the rat placed excessive stress on the thin film device and damaged the conductive traces to the head connector. While protection of the device in silicon was considered, it was determined that it would be insufficient and the implant should be constructed with minimal stress on any micro-fabricated, thin-film part of the implant. Examination of the device explanted from the initial experiments showed that the largest stress was placed on the section outside the spinal cord. As a result, it was determined that a bundle of wires should form connections between the head connector and the spinal baseplate. This baseplate would be used to interface between the wires and the microelectrode array, and fixing it to the spine would minimize the mechanical stress placed on the microelectrodes. Two implant configurations were considered as shown in **Figure 3**, and both used a baseplate with a fork that could be fixed to a spinous process. It was determined that the use of a U-turn places less stress on the array because motion of the spine is not amplified through a lever action. These considerations determined the basic implant layout illustrated in **Figure 2**.

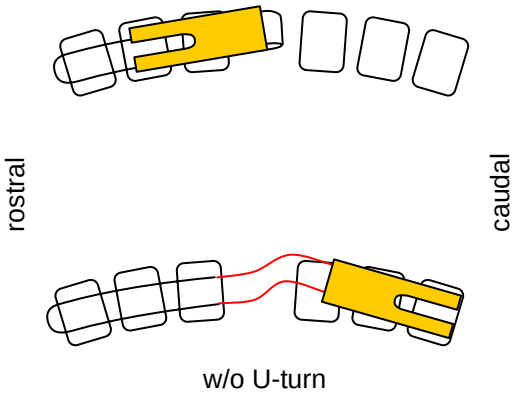


Figure 3. Two possible baseplate orientations.

Figure 4 shows two other design revisions. While there was moderate success with the design on the left, implant reliability was found to be a problem. Some devices showed partial or complete tears at the point marked B, although it was difficult to determine if the cause was the explantation procedure itself. Explants also showed considerable stress concentration at the points marked A and B, damaging the conductive traces inside the array. The design on the right of **Figure 4** shows modifications that were made to address these issues. A thicker silicone layer was preformed into the final shape and used around the U-turn (marked as C). Although this protective silicone layer could not be applied to the ventral side of the array without interfering with the electrode interface, it could be extended along the entire dorsal side.

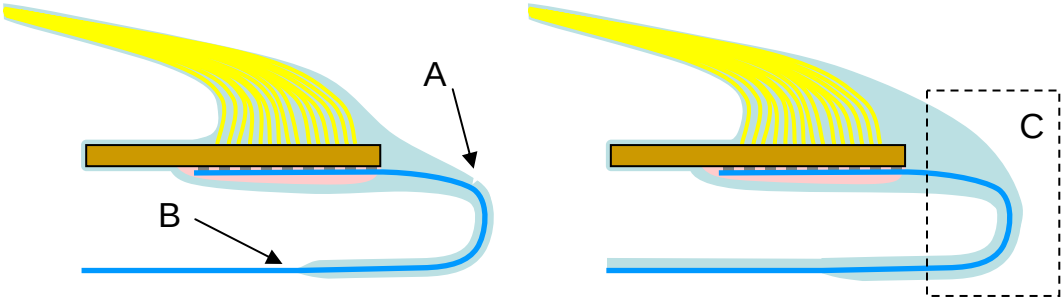


Figure 4. Intermediate designs. Areas of stress concentration (A and B) and improvements in the design (C).

Another problem observed with this implant design was that some rats lacked normal spinal reflexes for a few weeks after surgery. During explantation, it appeared that the thicker silicone made the implant stiffer in the region marked C in **Figure 4**, resulting in a spring effect that applied pressure on the spinal cord. **Figure 4C** show the current design, which involves a process that thins the protective silicone layer on the electrode array and uses a silicone overhang to protect the U-turn from any external pressure by surrounding soft tissue. Since the U-turn is much more flexible with this approach, it no longer applies undue pressure to the spinal cord. A finished device is shown in **Figure 5**.

Figure 5. Newest version of the chronic implant device

We also found that multiple wires implanted under the skin with regular motion between the head connector and the entry point into the spinal column can cause skin damage and ensuing infectious complications. As we increased the number of signal wires in our complex array designs, the bundle of wires that must be routed under the skin between the array terminus in the lumbar region of the spinal cord and the head connector became substantially larger. The wire bundle was sufficiently large and stiff such that it often chaffed against the skin of the animals when the animals were being trained or tested in the harness. To solve this problem we developed a new surgical technique for implanting the bundle of wires under the muscles to prevent direct contact between the wires and skin. Our recent experiments demonstrate that wires implanted in this manner remain stable, produce less damage to the skin, and reduce the risk of infection.

B. Microelectrode design

The implant structure is designed to minimize the mechanical stress placed on the microelectrode array during surgery implantation and normal motions of the rat. The most critical problem of the microelectrode design, however, is that during the post-implantation period of ~12 different implantations we have observed progressive electrode connectivity failures over a period of 2-6 weeks. Based on these experiments, we identified several specific factors underlying electrode failure, as well as solutions for each of these factors. We found that while some earlier designs of the arrays yielded moderate success, there was evidence of poor electrode adhesion to the parylene substrate (**Fig. 6)**.

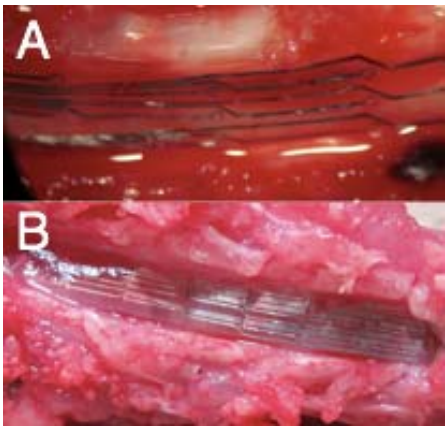


Figure 6. Delamination of electrodes. A. Example of detachments from the electrode pad base. **B.** Creasing of the parylene array substrate when implanted with silicone.

Initially the platinum on the electrodes was thickened using platinum electroplating to mitigate loss of the electrode during current injection, but it was found that this created a strong mechanical mismatch between the parylene substrate and the electrode surface (**Fig. 7).** This had a tendency to concentrate stress at the edges of the electrodes when the rat was moving, therefore electroplating was no longer used.

Figure 7. Mechanical mismatch causes stress at the electrode boundaries. Schematic illustrating electrode instability.

To solve this problem, several modifications have been or are being developed, and/or tested:
1. ***Argon-Ion etch of parylene prior to platinization.*** This additional process could provide additional cleaning of the parylene surface, and also provide additional free radicals at the surface, promoting better adhesion between the parylene and platinum layers. Surface treatment of the parylene beneath the platinum with argon plasma (as opposed to the usual oxygen plasma) was attempted but found to have no significant impact.
2. Thinner platinum layer. Reducing the electro-deposited layer will obviously lower the stress concentration effect. To compensate for changes in impedance, the platinum will be lightly doped to modify its electrical properties.
3. An adhesion layer of 100 angstroms of titanium on the parylene prior to platinum deposition. This additional process appears to be beneficial, as the most recent implants used arrays with this modification and have shown most electrodes to be functional well over one month after implantation, as opposed to the 2-3 weeks achieved with earlier designs.
4. Parylene surface grid. Parylene grid (2-μ) on top of the electrodes was design to help mechanically limit initiation and propagation of electrode delamination. In implants containing electrodes with and without such a grid, delamination was found more frequently on electrodes without the grid (**Fig. 8)**. These images show ripples in the platinum surface, but these are caused by exposure to hydrochloric acid used to dissolve tissues adhering to the implant after explantation.

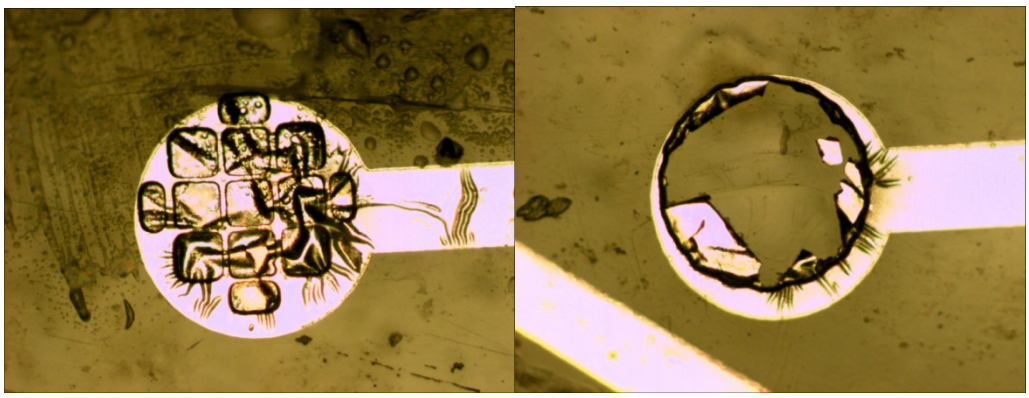


Figure 8. After being implanted for 8 weeks, electrodes with a protective parylene grid (left) were in better condition than electrodes without the grid (right) where most of the delamination was observed.

Further tweaks to the design will continue, as the traces running between the baseplate and each electrode have shown to break on occasion, resulting in 2-7 electrodes per implant gradually losing functionality. Nonetheless, the current design has shown satisfactory reliability, and progress towards the ideal will continue.

Aim 1.3: Develop a computer controlled 32-channel electrical stimulating system that can generate arbitrary sequences of neural simulation protocols that are needed for the array fabricated in Aim 1.1

One of goals of this research is provide a complete platform for spinal cord stimulation. In the short term this includes having computerized control of the stimulation, and in the longer term we hope to have most of the electronics implanted and embedded in the spinal baseplate. The latter will let us reduce the number of wires going to the head connector and possibly eliminate them altogether using a wireless design.

Figure 9 shows the layout and functioning prototype of a circuit that can route stimulation and recording signals from the implant to the appropriate data acquisition system. It is composed of 8:1 high voltage multiplexers (labeled M1-M9) that connect to the signals from the array as well as to the EMG signals, four shift registers at the top of the diagram used to configure the multiplexers with a serial data stream in under one microsecond, four low noise preamplifiers, and a few other power components (not shown). The circuit can be set up to stimulate or record between almost any two electrodes or amplify the signal from four electrodes or EMG wires simultaneously. The 27 electrodes, 2 ground wires, and 16 EMG wires (45 wires total) then are functionally reduced to one stimulation line and 4 amplifier signals, an enable signal, clock and data signals to configure the circuit, and power and ground lines (10 lines total). Because the circuit can be configured very rapidly, it can use time division multiplexing to record/stimulate more channels in a manner that is effectively simultaneous as far as the biology of the rat is concerned.

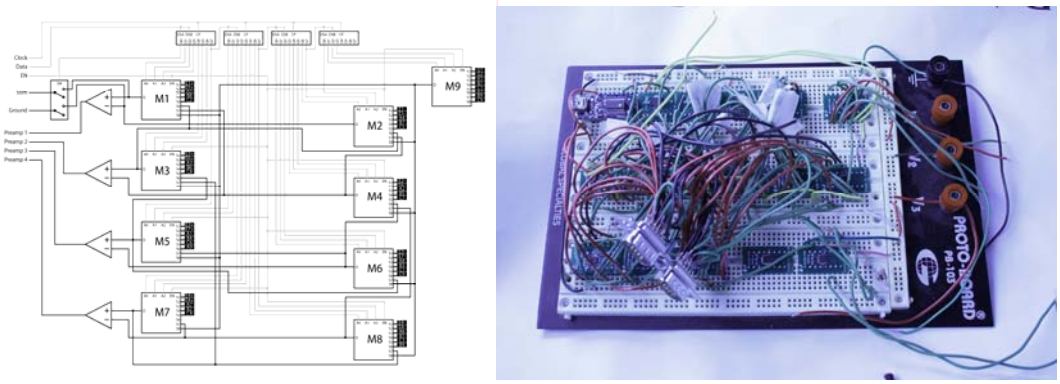


Figure 9. Multiplexer circuit and breadboarded prototype.

Custom software was written (**Fig. 10**) to interface with a data acquisition unit that outputs the correct serial data stream to configure the circuit as well as control the stimulation, and it was tested successfully when paired with the prototype multiplexer circuit.

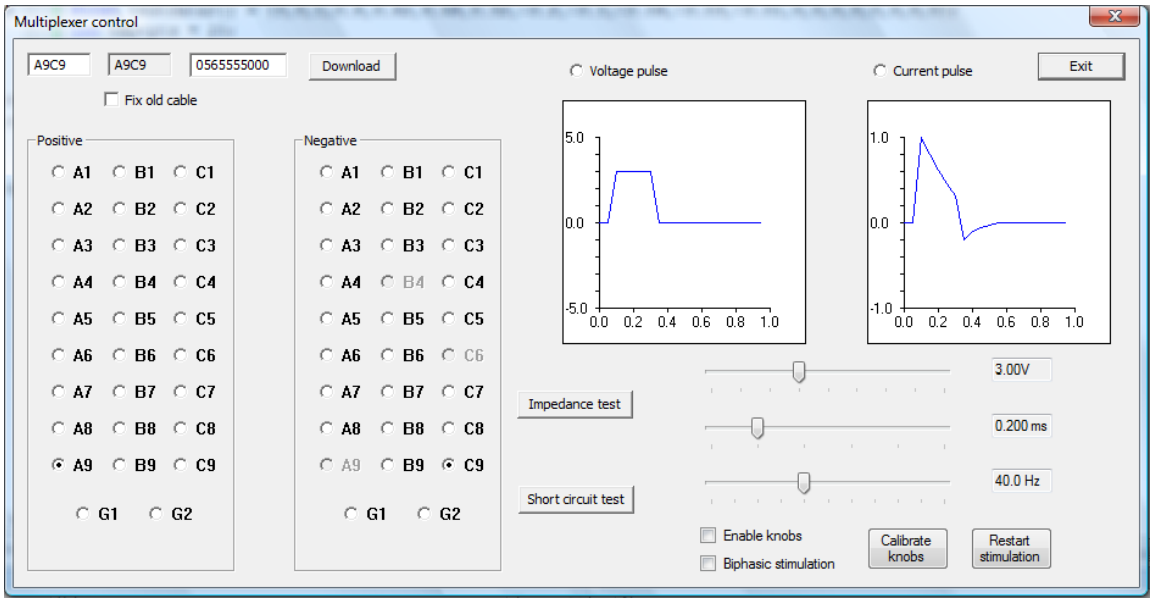


Figure 10. Multiplexer control software.

Aim 2: Characterize the basic properties of the stimulating array developed in Aim 1, and establish effective stimulation protocols for enabling stepping in SCI rats using these high-density EAs.

We have tested basic properties of the stimulating array developed in Aim 1, during acute and chronic experiments.

During acute, non-survival experiments on continuously anesthetized (isoflurane gas) rats, ES arrays were implanted on the lumbosacral spinal cord. By stimulating selected electrodes on the array and recording from the array during peripheral nerve stimulation, we demonstrated that the array could be successfully used to monitor muscle and spinal cord potentials. The spinal somatosensory-evoked potentials measured at several levels of the spinal cord were successfully recorded from normal, uninjured rats. Motor-evoked potentials from stimulation at different spinal segments were successfully recorded from selected muscles on the same rats and correspond to our previous data collected when stimulating with conventional electrodes (Gerasimenko et al., 2006; Lavrov et al. 2006) and with previous array designs. Monitoring of spinal cord potentials at different time points after the injury and treatment initiation will provide insight into the synaptic mechanisms that are involved in the recovery of motor functions and their reorganization after a spinal cord injury.

During the chronic experiments several stimulation paradigms to facilitate stepping in the spinal rats were tested, i.e., monopolar stimulation (between a local electrode and a distant indifferent electrode), bipolar stimulation (stimulation across a single pair of local electrodes), combined monopolar (between several local electrodes and a distant electrode), and combined bipolar stimulation (stimulation across several pairs of local electrodes). Our results suggest that selected bipolar, but not monopolar, stimulation of spinal cord facilitates bipedal hindlimb stepping with plantar placement in spinal rats within 1-2 weeks post-injury. In comparison monopolar stimulation on the same animals facilitated stepping activity at ~3 weeks after injury. We found that, at this early time frame, stimulation of the caudal spinal segments facilitated stepping patterns, while stimulation of the rostral segments produced only tonic responses. **Figure 11** shows the results of stimulating the dorsal cord using various combinations of bipolar vs. monopolar stimulation configurations, as well as the effect of more ventral vs. more caudal stimulation foci. As seen in the step kinematics diagrams in both columns, stimulation through some combinations of electrodes can elicit active stepping. Stepping recovery was observed within 1.5 weeks of initial implantation of the arrays after the spinal cord injury, vs. 2.5 weeks after the injury with conventional wire stimulating electrodes.

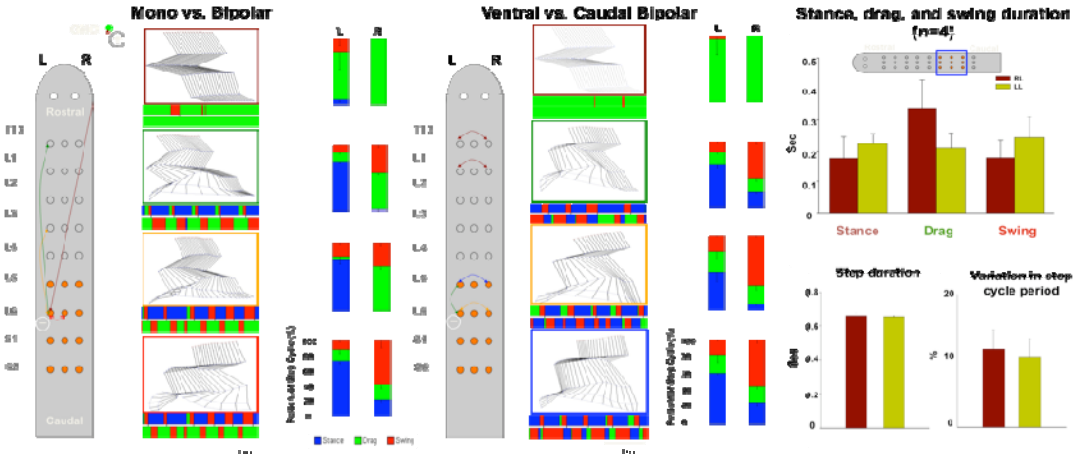


Figure 11: Effect of selective epidural stimulation with multi-electrode array system.

Aim 3: Test the hypothesis that high density ES, coupled with robotically-guided physical training will result in better stepping performance than ES alone or robotic training alone.

As we mentioned earlier the only negative outcome of this project is that we were not been able to completely accomplish SA3 because technical limitation and delay in array fabrication. Now we have all required technologies and tools to complete the last goal and determine whether stepping can be best achieved by combining daily periods of ES with electrode array in combination with step training. We plan to accomplish the following goals to complete SA3:

1. **Test the improved array design and multiplexer system in chronic *in vivo* studies.** We are planning to implant one group of spinal cord injured rats with the improved design and another group with the next generation of the array with the multiplexer system.

2. **Test the hypothesis that stimulation with high-density epidural stimulating arrays, coupled with robotically guided physical training will result in better stepping performance than with epidural stimulation alone or robotic training alone.** Based on our previous studies we expect earlier stepping recovery and accordingly earlier initiation of step training with electrode array (within 1.5 weeks using the array system vs. 2.5 weeks with conventional electrode). We also expect to see a strong synergistic effect between epidural stimulation with the array and robotic-assisted step training, resulting in improved recovery of stepping ability (as measured by the number of steps, reduced variation in the stepping patterns, and better coordination and timing of the stepping patterns) than either therapy alone. As originally proposed (only later than anticipated) we will test our hypothesis on chronically implanted complete spinal rats and we will compare the level of locomotor recovery among rats in the following groups: a) rats trained with a combination of epidural stimulation and step training, b) rats receiving only epidural stimulation, and c) rats receiving only robotically guided locomotor training.

KEY RESEARCH ACCOMPLISHMENTS:

- We have designed and fabricated the next generation of 3x9 flexible ES array system.
- The arrays' mechanical and electrical properties were bench-top tested.
- A flexible ES array was successfully interfaced with multichannel connectors, an extension cable, and a multichannel headplug developed for the rat model.
- We have been able to maintain functional implanted arrays in complete spinal rats for 6 weeks.
- We found stable parameters of some electrode arrays (impedance of electrodes and thresholds of evoked responses) for up to 6-8 week period.
- We observed stable rhythmic hindlimb activity facilitated with stimulation from selected electrode combinations after a complete mid-thoracic spinal cord transection.
- We have identified new machine learning paradigms that have the potential to find the optimal array stimulation parameters more rapidly, as well as adapt the array stimulation to individual animals.

REPORTABLE OUTCOMES:

Papers

1. Gad P, Woodbridge J, Lavrov I, Gerasimenko Y, Zhong H, Roy RR, Sarrafzadeh M, and Edgerton VR. Electronic spinal bridge to facilitate stepping after a complete spinal cord lesion. Journal of Neural Engineering, 2010.
2. Fong AJ, Roy RR, Ichiyama RM, Lavrov I, Courtine G, Gerasimenko Y, Tai YC, Burdick J, Edgerton VR. Recovery of control of posture and locomotion after a spinal cord injury: solutions staring us in the face. Prog Brain Res. 175: 393-418. Review, 2009.

Abstracts

1. Gad P, Woodbridge J, Lavrov I, Gerasimenko Y, Zhong V, Roy RR, Sarrafzadeh M, Edgerton VR. Development of an electronic spinal bridge between the forelimbs and hindlimbs to facilitate quadrupedal stepping after a complete spinal cord transection. Society for Neuroscience, 2010.
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3. Lavrov I, Nandra M, Choe J, Gad P, Dy C.J, Fong A.J, Tai Y.C, Burdick J.W, Zhong H, Roy R.R, and Edgerton V.R. Spinal cord stimulation with high-density flexible multi-electrode array improves stepping facilitation after a complete spinal cord injury. *Dynamics of Neural Microcircuits Symposium, UCLA, 2009*

Patents

1. High density epidural stimulation for facilitation of locomotion recovery after Spinal cord injury. Victor R Edgerton, Roland R. Roy, Igor Lavrov, Yury Gerasimenko US Patent. Assignee UC. Case No. 2010-470-1.

CONCLUSION:

All data are supportive that the new flexible electrode array design can potentially improve the capacity to stand and step in spinal animals and in individuals with severe SCI. This new design will also provide essential neurophysiological information regarding the assessment of spinal cord circuitry after the injury. Utilizing the currently available electrode array design will help to achieve our final goals, i.e. test on animal models a combination with training and pharmacological treatments, and ultimately transfer it for clinical use in humans.

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4. Gad P, Woodbridge J, Lavrov I, Gerasimenko Y, Zhong V, Roy RR, Sarrafzadeh M, Edgerton VR. Development of an electronic spinal bridge between the forelimbs and hindlimbs to facilitate quadrupedal stepping after a complete spinal cord transection. Society for Neuroscience, 2010.
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APPENDIX: N/A